Original Article

Pharmacology and molecular docking study of cartilage protection of Chinese herbal medicine Fufang Shatai Heji (STHJ) by inhibiting the expression of MMPs in collagen-induced arthritis mice

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Background: This study aims to explore whether Fufang Shatai Heji (STHJ), as a mixture collected by a decoction of a variety of Chinese herbal medicines for immune system diseases, can improve the cartilage destruction of rheumatoid arthritis (RA).

Methods: The therapeutic effects of STHJ were studied using collagen induced arthritis (CIA) mice. The improvement effect of STHJ on synovitis and cartilage damage caused by arthritis was studied by joint pathological analysis. The inhibitory effect of STHJ on related degradation enzymes in cartilage was studied by immunohistochemistry and real-time polymerase chain reaction (PCR). The specific targets of STHJ were predicted by molecular docking.

Results: After successfully inducing CIA, the paws of the mice showed significant swelling, and athological analysis of the ankle and knee joints also showed significant cartilage destruction and synovial hyperplasia. However, synovial hyperplasia and cartilage destruction were markedly alleviated after administration of STHJ. And after STHJ treatment, the expression of ADAMTS-4, ADAMTS-5, MMP-9 and MMP-13, in the cartilage layer of CIA mice was significantly inhibited. Through molecular docking assays, we proved that acteoside in STHJ could directly bind to the Glu111, Phe110 residues in MMP-9 and glycyrrhizic acid in STHJ bind to the Glu382, Asn433 residues in MMP-13.

Conclusions: Our results suggested that STHJ may alleviate synovial hyperplasia and cartilage destruction in CIA mice and protect cartilage by inhibiting the expression of MMP-9 and other enzymes.

Keywords: Fufang Shatai Heji (STHJ); collagen-induced arthritis (CIA); cartilage; MMPs; ADAMTS

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune problem in modern world. The disease features in severe synovitis and the progressive destruction of joint structures (1). Collagen-induced arthritis (CIA) mouse model created in 1980 which was induced by type II collagen (CII) has become one of the most widely used models in RA (2). CIA is relatively easy to induce in

genetically susceptible mouse strains by immunization, and synovitis and bone erosion in disease progression are similar to RA (3). Therefore, we used CIA as an animal model of RA in this study. The course of RA is very long and if effective treatment is not available, the destruction of bones and cartilage will eventually lead to joint deformities and dysfunction, and even physical disabilities (4). Among the current treatments, most drugs have no significant effect in controlling bone damage caused by RA. For example, long-term use of methotrexate and dexamethasone can induce osteoclast accumulation leading to further bone destruction (5,6). Therefore, the development of drugs that inhibit bone and cartilage destruction is particularly essential in enhancing RA treatment.

Fufang Shatai Heji (STHJ), the gathered liquid collected from secondary decoction of diverse herbs, mainly includes *Glycyrrhiza Uralensis*, *Radix Ophiopogonis*, *Radix Astragali*, *Radix Pseudostellariae*, *Radix Adenophorae*, *Rehmannia glutinosa*, *Triticum aestivum*, *Prunella vulgaris* and *Dendrobium nobile*. Astragaloside IV is the main component of STHJ, and its content determination is used as a quality control method for STHJ (7,8). Calycosin (CAL) in *Radix Astragali* had a significant inhibitory effect on proinflammatory factors secreted by rheumatoid arthritis synovial fibroblasts (RASFs) (9). Catalpol in *Rehmannia glutinosa* can promote the osteogenic differentiation (10,11). Ophiopogon D in *Radix Ophiopogonis* could reduce the mRNA expression of osteoclast gene to protect the bone destruction in mice (12). Flavonoids in *Glycyrrhiza Uralensis* significantly reduced chronic inflammation and inflammatory pain in RA (13). Furthermore, our previous studies demonstrated that STHJ has effect in improving immune function in mice (7). In our previous study, STHJ can significantly regulate the metabolism of $CD4^+$ T, $CD8^+$ T, $CD8^+CD122^+$ T, NKT, γδT, B and natural killer (NK) cells to regulate the body's immune function. Moreover, our previous study also found that STHJ can improve the spleen damage of CIA mice (14). Therefore, we suspect that STHJ may be effective against CIA mice.

In this research, we explored the potential benefits of STHJ on the joints of CIA mice. The mechanism of cartilage protection of STHJ was studied in the ankle joint, knee joint and femoral head of CIA mice. The binding site of STHJ in RA cartilage was further studied through molecular docking assay.

We present the following article in accordance with the ARRIVE reporting checklist (available at https://apm.amegroups.com/article/view/10.21037/apm-21-1765/rc).

Methods

Human protein-protein interaction (PPI) network related to RA

In order to study the possible mechanism of STHJ in the treatment of RA, we use data mining methods to study the PPI network related to RA. STRING (http://string-db.org/) is a website for evaluating interacting genes/proteins. According to the information in the STRING database (PPI score >0.7), the protein (species: human) related to the differentially expressed gene (DEG) was selected, and then the PPI network was established using Cytoscape software (http://cytoscape.org/).

Animals

DBA/1J mice (male, 20–25 g, 6–8 weeks old) were purchased from Shanghai SLAC Animal Center (Shanghai, China) remained placed in a special environment which were SPF facilities with specific-pathogen-free facilities. The Animal Ethics Committee of Shanghai Ninth People's Hospital approved all animal experimental protocol (No.: SH9H-2021-A580-1), and the experiment complies with the institution's guidelines on the care and use of animals.

Drugs and reagents

Shanghai Ninth People's Hospital (Shanghai, China) provided STHJ. Two mg/mL bovine type II collagen, 10 mg/mL Complete Freund's Adjuvant were provided by Chondrex (Woodinville, USA). The primary antibodies of MMP-9, MMP-13, and ADAMTS-5 were provided by Cell Signaling Techology (Danvers, USA). The cDNA reverse transcription kit was provided by Takara Bio (Kusatsu, Japan). TRIzol reagent was acquired from Ambion (Shanghai, China).

Experimental protocols

Thirty mice were aimlessly divided into three groups with 10 in each. The three groups were treated differently. One was the normal group; the other two were induced by intradermal injection of 1:1 mixed CII (2 mg/mL) and CFA at the bottom of the tail (0.1 mL per) on day 0 and day 21 respectively (*Figure 1*). After successful modeling, the STHJ group (10 mL/kg) was infused with STHJ by intragastric gavage once a day for 35 consecutive days. The dose of STHJ is calculated based on the effective dose for

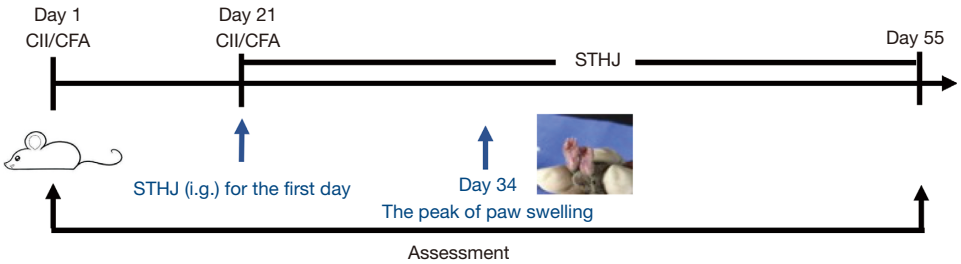

Figure 1 Graphical protocol for collagen-induced arthritis (CIA) induction and Fufang Shatai Heji (STHJ) administration and the effect of STHJ on CIA mice. DBA/1J mice were immunized with collagen II and complete Freund's adjuvant (CII/CFA) (0.1 mL per) on day 0 and 21. The mice in the treatment group were infused with STHJ (10 mL/kg) intragastrically every day for 35 consecutive days after modeling.

long-term clinical use. The model group were conducted by normal saline in the same way.

Overall efficacy evaluation

From the beginning of the first immunization, two independent observers examined the body weight and the severeness of arthritis twice a week. The arthritis severity index was adopted to record the severity of arthritis, with a score of 0–4 (15). 0, normal; 1, mild swelling; 2, moderate swelling; 3, obvious swelling; 4, severe arthritis.

Histopathological analysis of joints

At the end of the experiment, sodium pentobarbital (45 mg/kg) was used to anesthetize all mice by intraperitoneal injection and then killed by cervical dislocation. The hind legs were carefully disassociated and settled in 4% paraformaldehyde (PFA) for 24 hours, then decalcified and encapsulated in paraffin. The joint tissues were routinely sectioned, dewaxed with xylene, dehydrated by ethanol gradient, and then tinted by hematoxylin and eosin (H&E), alcian blue and orange G. Semi-quantitative scoring of inflammatory cell infiltration, synovial liner cell proliferation and cartilage damage was conducted by two independent observers. The inflammatory activity score was recorded on a 0–4 scale as followed: 0, normal; 1, mild infiltration with no proliferation of synovial liner cells; 2, moderate infiltration with proliferation of some synovial liner cells; 3, marked infiltration with synovial liner cells hyperplasia; 4, severe infiltration with remarkable synovial liner cells.

Immunohistochemistry

Tissue sections were deparaffinized in xylene and then hydrated with gradient alcohol. After antigen retrieval, they were incubated with the corresponding primary antibody overnight at 4 ℃. Wash off the primary antibody and incubate the secondary antibody for 30 minutes, then counterstained with Mayer hematoxylin. Two independent researchers observed the positive cells under a microscope.

Real-time polymerase chain reaction (PCR)

Use TRIzol reagent to obtain total RNA from mouse femoral head. Reverse transcription was performed using the cDNA reverse transcription kit. Real-time quantitative PCR primers were purchased from Shanghai Shenggong Biological Engineering Technology Service Co., Ltd. The primers were exhibited in *Table 1*. Quantitative PCR was executed on the real-time PCR system (Thermo Fisher Scientific, IL) conforming to the corporation's information. The operation using the PCR cycler was as follows: prenotation at 95 ℃ for 30 min, 40 cycles of denaturing at 95 ℃ for 5 sec and replication at 60 ℃ for 34 sec. Normalized the PCR data to the level of β-actin gene expression and calculated the value by the following formula: $2^{-\Delta\Delta ct}$.

Molecular docking

The active ingredients in STHJ were screened using the Traditional Chinese Medicine Systems Pharmacology (TCMSP, https://tcmspw.com/tcmsp.php) database. Auto

Table 1 Primer sequences in real-time polymerase chain reaction (PCR)

Gene	Upstream primer	Downstream primer
MMP-9	5'-GCAGAGGCATACTTGTACCG-3'	5'-TGATGTTATGATGGTCCCACTTG-3'
ADAMTS-4	5'-ATGGCCTCAATCCATCCCAG-3'	5'-AAGCAGGGTTGGAATCTTTGC-3'
ADAMTS-5	5'-GGAGCGAGGCCATTTACAAC-3'	5'-CGTAGACAAGGTAGCCCACTTT-3'
Aggrecan	5'-CCTGCTACTTCATCGACCCC-3'	5'-AGATGCTGTTGACTCGAACCT-3'
Type X collagen	5'-GGGACCCCAAGGACCTAAAG-3'	5'-GCCCAACTAGACCTATCTCACCT-3'

Dock 4.2.6 program was used for molecularly dock the active ingredients in STHJ with potential protein targets. The selection of docking parameters is assigned according to the strategy proposed in the parameter testing section, and default values are used for all other parameters not mentioned. In this study, the active ingredients with binding energy ≤–5.0 kJ/mol were selected as the screening basis for STHJ to bind RA potential targets.

Statistical analysis

All data are evinced as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) in SPSS 22.0 software (IBM, New York, USA) was used to analyze statistical differences between various groups. A possibility of $P<0.05$ was thought statistically cogent.

Results

Analysis of key genes in PPI network

The new network consists of 269 genes, representing the main target genes regulated in the treatment of RA. In addition, some genes such as MMP9, MMP13, ADAMTS-5 are found to be important genes related to the development of RA (*Figure 2*). Therefore, these genes may also be the main target genes for STHJ to treat RA.

Protective effect of STHJ on CIA mice

No mouse death was observed throughout the experiment. Compared with the control group (*Figure 3A*), CIA mice lose weight obviously ($P<0.01$) while no significant improvement in body weight was observed in the STHJ-treated group in comparison with the CIA group. From day 0 to day 55, the arthritis score and paw swelling of each group of mice were evaluated (*Figure 3B*,*3C*). CIA mice showed significant arthritic symptoms on day 28 and gradually reduced symptoms from day 42. The paws swelling of STHJ-treated mice was significantly reduced from day 34, and the arthritis score was significantly reduced from day 49. *Figure 3B* showed that increasing values of all three groups on day 55 may be caused by external factors such as machines and personnel. Photographs of the morphology of the hind paw of mice on day 55 was showed in *Figure 3D*. Compared with the control group, the hind paws of CIA mice were much more bloated, and STHJ significantly improved the degree of swelling.

STHJ inhibited ankle and knee joint damage in CIA mice

The CIA mice showed significant inflammatory infiltration, synovial hyperplasia and cartilage destruction in the ankle joints, and this phenomenon was significantly improved after STHJ treatment (*Figure 4*). Synovial inflammation score and chondrocyte semi-quantitative analysis further showed that STHJ can significantly inhibit synovial inflammation and cartilage destruction in the ankle joints.

Compared with the control group (*Figure 5*), inflammatory infiltration, synovial hyperplasia and cartilage destruction occurred in the knee joints of CIA mice, and proteoglycan consumption and chondrocyte reduction were observed. The results of semi-quantitative analysis also showed that the cartilage layer became thinner, the area of cartilage was reduced, and chondrocytes were severely lost in the knee joints of CIA mice. After STHJ treatment, significant alleviation appeared in synovial inflammation and cartilage destruction.

STHJ inhibited expression of related degrading enzymes in articular cartilage of the knee and ankle joints in CIA mice

The previous PPI study showed that MMPs and ADAMTS

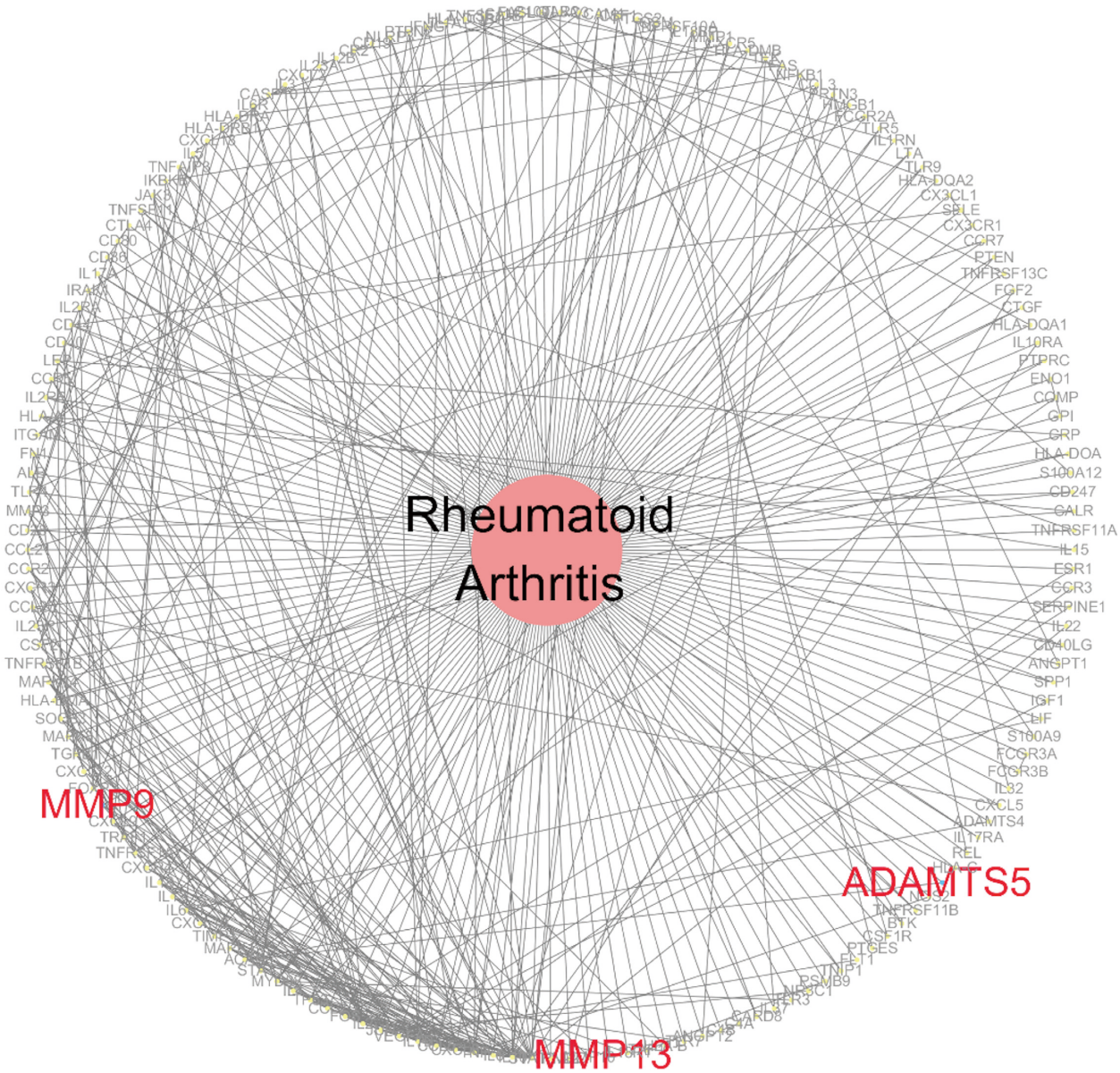

Figure 2 The protein-protein interaction (PPI) network of potential targets in rheumatoid arthritis (RA). The more evidence of interaction, it means the importance of related nodes.

play an important role in the joint damage of RA. Therefore, we studied the effect of STHJ on the expression of MMPs and ADAMTS in cartilage.

The results of immunohistochemistry experiments of the ankle joint showed that the expression of MMP-9, MMP-13 and ADAMTS-5 increased significantly, but decreased significantly after STHJ treatment (*Figure 6*). The results of immunohistochemistry experiments of the knee joint showed that the expression of MMP-9, MMP-13 and ADAMTS-5 increased significantly, and decreased significantly after STHJ treatment. However, STHJ has a more significant inhibitory effect on the expression of MMP-9 and MMP-13 in the ankle joint compared with knee arthritis (*Figure* 7).

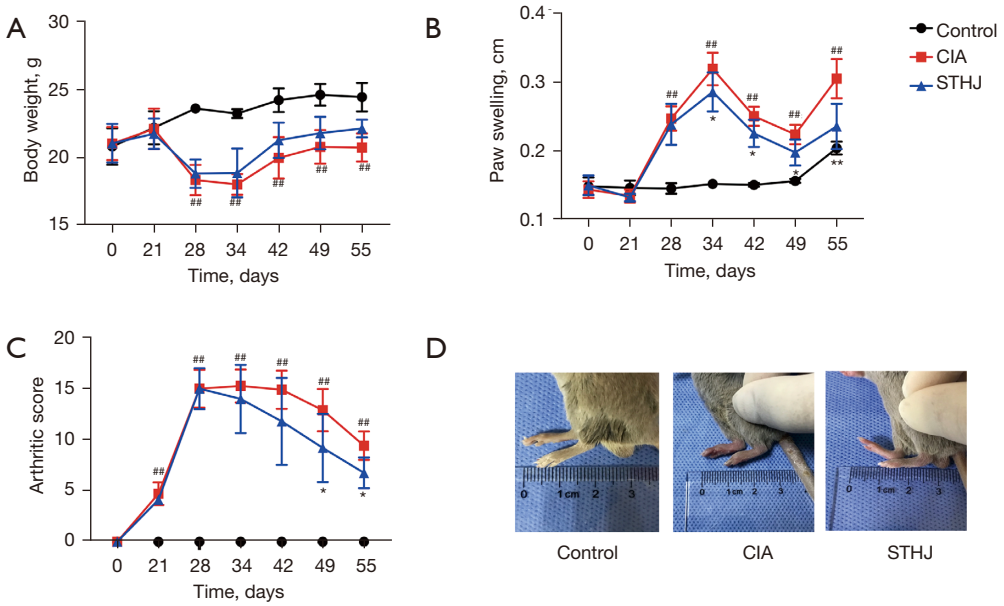

Figure 3 Protective effect of Fufang Shatai Heji (STHJ) on collagen-induced arthritis (CIA) mice. (A) Changes in body weight of different groups. CIA mice showed obvious weight loss from day 28 and there was no significant improvement after STHJ treatment. (B-D) The paw swelling and arthritic scores of the mice. The CIA group showed obvious swelling of the ankle joint from day 28. After STHJ administration, the swelling of the ankle joint could be obviously alleviated from day 34, and the longer the effects were more obvious. Discovered from clinical scores, the arthritis symptom of the CIA group was found on day 21, and obvious from day 28. After STHJ administration, arthritis symptom was significantly relieved from day 49. All data are represented as mean ± standard deviation (SD). ##, P<0.01 *vs.* the control group; *, P<0.05, **, P<0.01 *vs.* the CIA group.

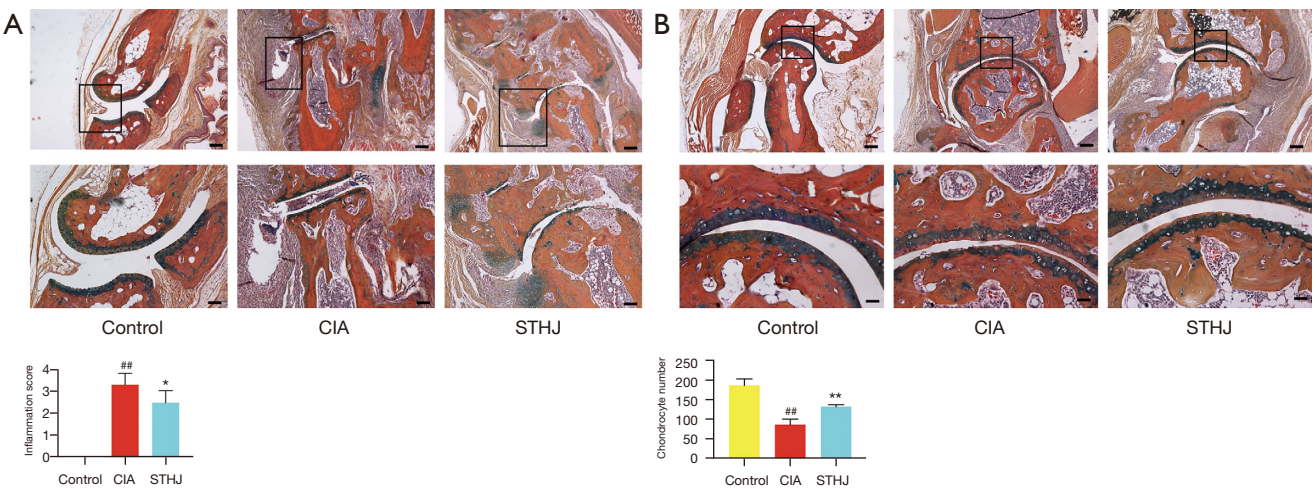

Figure 4 Effect of Fufang Shatai Heji (STHJ) on the synovium (A) and cartilage (B) of the ankle joints of the control, collagen-induced arthritis (CIA), and STHJ group. The hind legs are encapsulated in paraffin after fixing and decalcification. Routine sections of joint tissues were stained with alcian blue, hematoxylin and eosin (H&E), and orange G. The black box represents the corresponding enlarged area in the figure below. Severe synovial hyperplasia and cartilage destruction occurred the ankle joints of CIA mice and was significantly reduced after STHJ treatment. Scale bar, 200 μm (upper panel) and 100 μm (lower panel). All data are represented as mean ± standard deviation (SD). ##, P<0.01 *vs.* the control group; *, P<0.05, **, <0.01 *vs.* the CIA group.

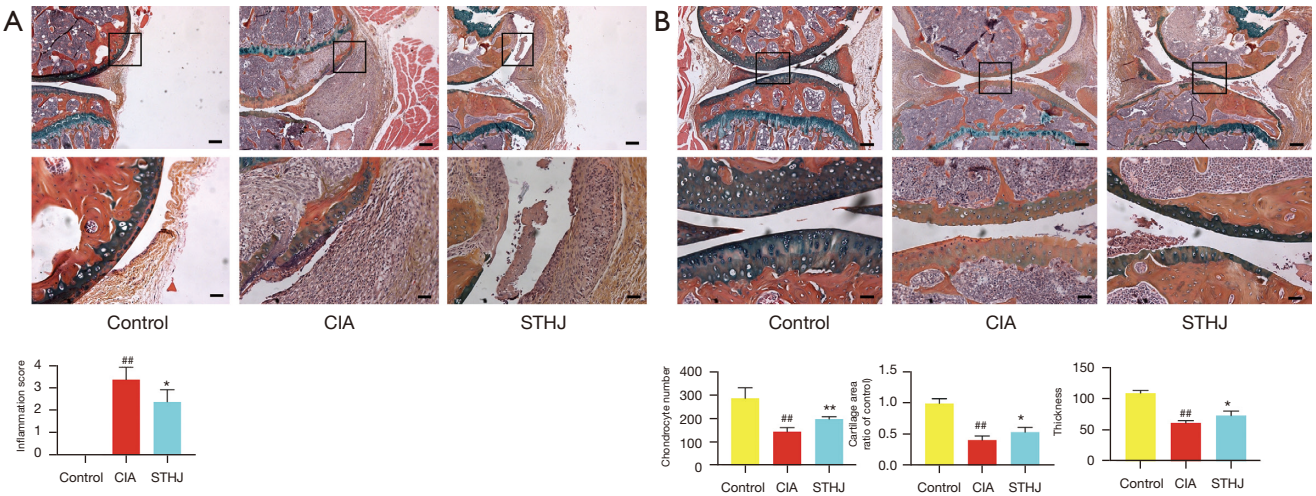

Figure 5 Effect of Fufang Shatai Heji (STHJ) on the synovium (A) and cartilage (B) of the knee joints of the control, collagen-induced arthritis (CIA), and STHJ group. The hind legs are encapsulated in paraffin after fixing and decalcification. Routine sections of joint tissues were stained with alcian blue, hematoxylin and eosin (H&E), and orange G. The black box represents the corresponding enlarged area in the figure below. The synovial membrane was hyperplasia severely, and chondrocytes and extracellular matrix were severely lost in the knee joint of CIA mice, but was significantly inhibited after STHJ treatment. Scale bar, 200 μm (upper panel) and 100 μm (lower panel). All data are represented as mean ± standard deviation (SD). ##, P<0.01 *vs.* the control group; *, P<0.05, **, P<0.01 *vs.* the CIA group.

Effect of STHJ on the expression of related genes in cartilage

The mRNA expressions of MMP-9, ADAMTS-4, ADAMTS-5, Aggrecan, and type X collagen in cartilage were evaluated by RT-PCR. The expression of ADAMTS-4, ADAMTS-5, MMP-9, and type X collagen in CIA mice was increased significantly but reduced after STHJ administration (*Figure 8*). The mRNA expression of aggrecan in the cartilage of CIA mice was significantly reduced but increased after STHJ administration (*Figure 8*). Furthermore, the inhibition of STHJ on ADAMTS-4, ADAMTS-5 was more obvious.

STHJ bound to MMPs

STHJ can indeed inhibit disease progression by reducing the expression of MMPs and ADAMTS, but the specific target is still unknown. Therefore, we used molecular docking studies to predict possible targets. Thirteen active ingredients were screened from the 12 medicinal materials in STHJ through the TCMSP database. *Figure 9* showed the docking results of chemical components with MMP-9, MMP-13 and ADAMTS-5. A variety of active ingredients in STHJ have good binding activity with potential targets (*Table 2*). Acteoside directly binds to Tyr423, Glu111, Phe110, Ala191 and Ala189 residues in MMP-9 (*Figure 9A*). Glycyrrhizic acid directly binds to residues Glu384, Asn433, Lys432, Glu333, Gly292 and Ser289 in MMP-13 (*Figure 9B*). However, taraxerone and ADAMTS-5 do not have good binding activity (*Figure 9C*).

Discussion

The major disease characteristics of RA is sustained synovial inflammation, which then destroys bone and cartilage (16). Chronic synovial inflammation can cause joint swelling, while cartilage and bone destruction can lead to joint malformation and even dysfunction (17,18). Articular cartilage is a layer of avascular, non-neural, terminally differentiated tissue covering the surface of the joint bone, mainly consist of extracellular matrix (ECM) and chondrocytes. Chondrocytes are the onliest cellular components in articular cartilage that support the balance of articular cartilage and ECM. ECM is mainly made up of tissue fluid, collagen, and proteoglycan. Under normal circumstances, the equilibrium of synthesis and degradation of ECM maintains the homeostasis of cartilage (19). However, this balance is disrupted in RA, where matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin-like motifs

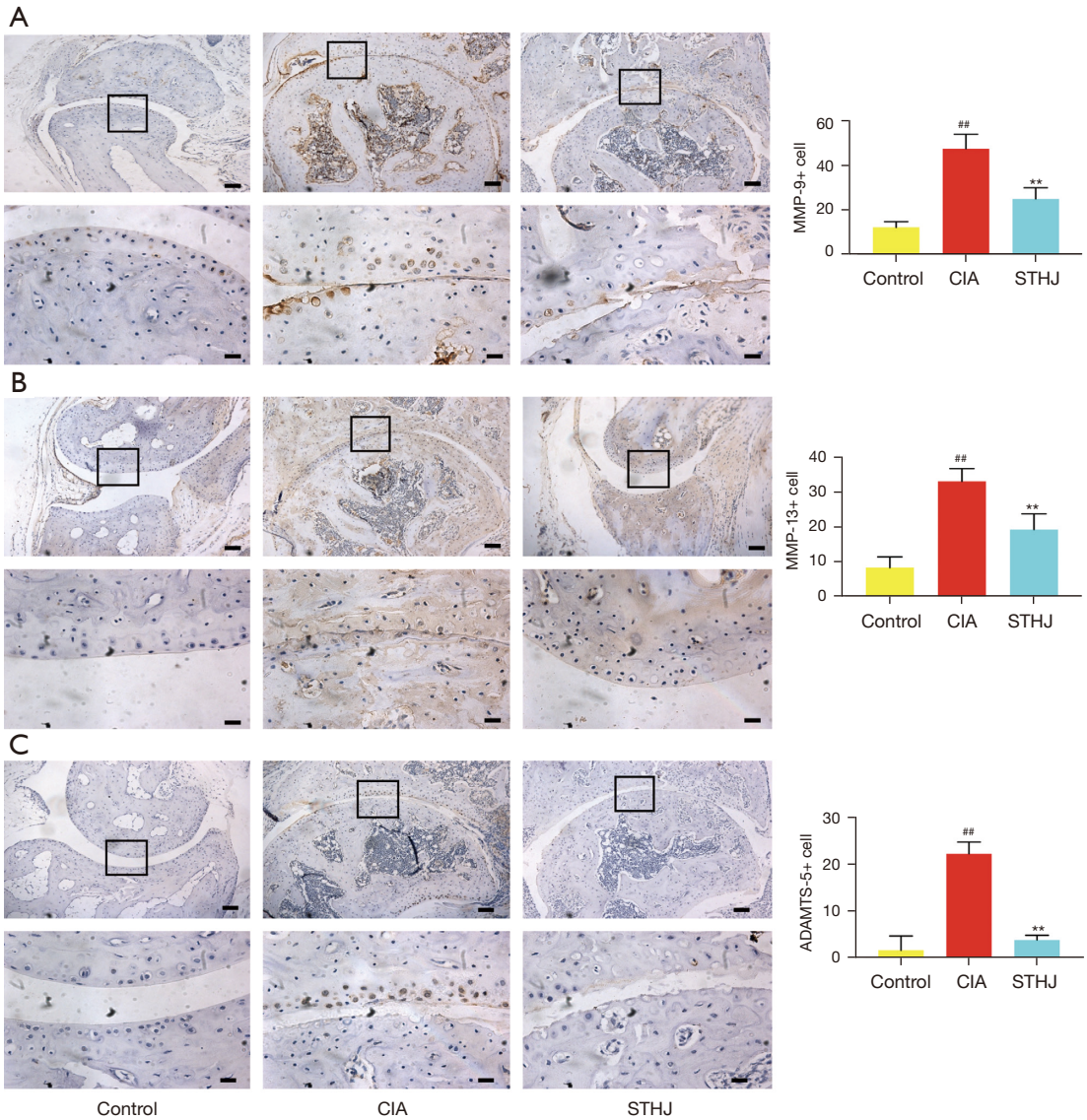

Figure 6 Effect of Fufang Shatai Heji (STHJ) on the expression of MMP-9 (A), MMP-13 (B) and ADAMTS-5 (C) in the ankle cartilage of the control, collagen-induced arthritis (CIA), and STHJ group. Tissue sections were deparaffinized in xylene and then hydrated with gradient alcohol. After antigen retrieval, they were incubated with the corresponding primary antibody overnight at 4 °C. Wash off the primary antibody and incubate the secondary antibody for 30 minutes, then counterstained with Mayer hematoxylin. The black box represents the corresponding enlarged area in the figure below. Compared with the control group, the expression of MMP-9, MMP-13 and ADAMTS-5 was significantly increased in the ankle cartilage of CIA group. After STHJ administration, the expression in cartilage was obviously reduced. Scale bar, 200 μm (upper panel) and 50 μm (lower panel). All data are represented as mean ± standard deviation (SD). ##, P<0.01 *vs.* the control group; **, P<0.01 *vs.* the CIA group.

(ADAMTS) are thought to act a pivotal part (20). Besides, the iaggression of inflammatory synovial cells and enlivening of fibroblast-like synoviocytes leads to the release of a wide range of inflammatory factors that motivate the production of ADAMTS and MMPs by chondrocytes or synovial cells, further exacerbating damage to the articular cartilage (21,22). Therefore, the development of effective drugs to protect bone and cartilage is of vital importance during RA treatment.

The mechanism of STHJ on RA is unclear. We first

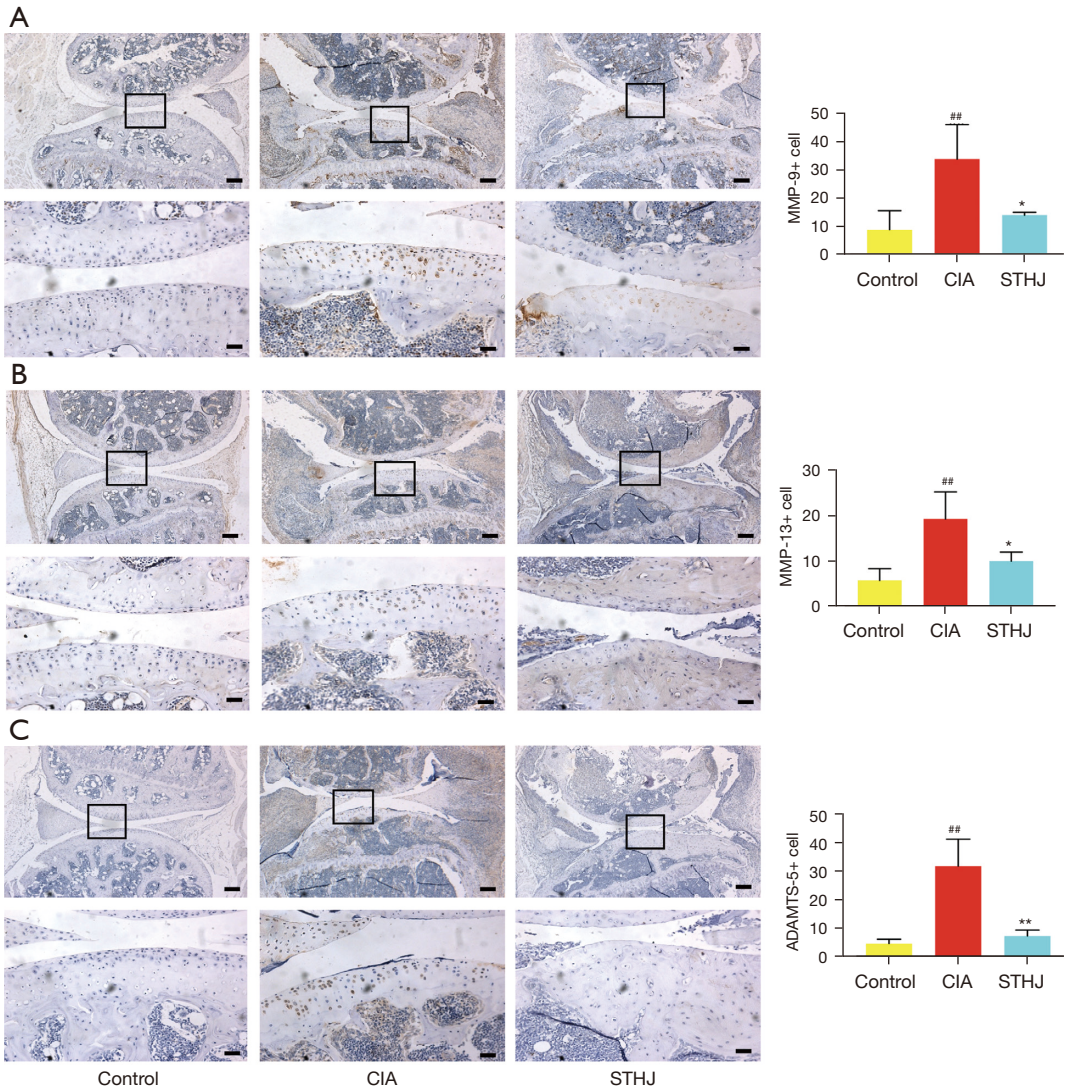

Figure 7 Effect of Fufang Shatai Heji (STHJ) on the expression of MMP-9 (A), MMP-13 (B) and ADAMTS-5 (C) in the knee cartilage of the control, collagen-induced arthritis (CIA), and STHJ group. Tissue sections were deparaffinized in xylene and then hydrated with gradient alcohol. After antigen retrieval, they were incubated with the corresponding primary antibody overnight at 4 °C. Wash off the primary antibody and incubate the secondary antibody for 30 minutes, then counterstained with Mayer hematoxylin. The black box represents the corresponding enlarged area in the figure below. Compared with the control group, the expression of MMP-9, MMP-13 and ADAMTS-5 was significantly increased in the ankle cartilage of CIA group. After STHJ administration, the expression in cartilage was significantly reduced. STHJ has a more significant inhibitory effect on the expression of MMP-9 and MMP-13 in the ankle joint compared with knee arthritis. Scale bar, 200 μm (upper panel) and 100 μm (lower panel). All data are represented as mean ± standard deviation (SD). ##, P<0.01 *vs.* the control group; *, P<0.05, **, P<0.01 *vs.* the CIA group.

found that MMP-9, MMP-13 and ADAMTS-5 play potential targets in the progression of RA through the protein-protein interaction (PPI) network. Next, we would verify the specific mechanism of STHJ on the CIA animal model. Histological staining analysis of knee and ankle joints of CIA mice revealed that the cartilage layer was damaged and the chondrocytes were severely lost. However, the cartilage destruction was significantly suppressed after STHJ treatment. Endochondral ossification is one of the important reasons for cartilage destruction. During

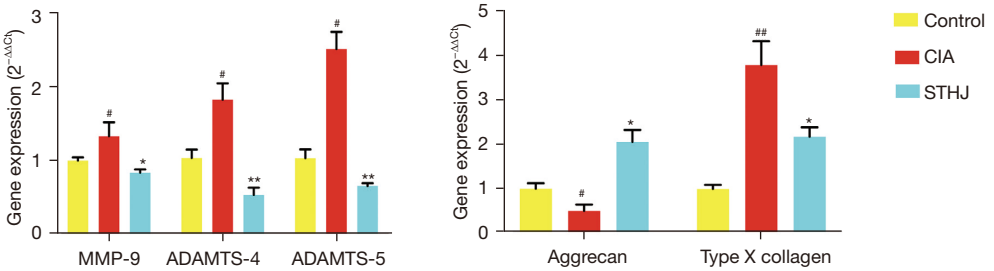

Figure 8 The messenger ribonucleic acid (mRNA) expression of ADAMTS-4, ADAMTS-5, MMP-9, aggrecan, type X collagen in cartilage. The expression of ADAMTS-4, ADAMTS-5, MMP-9, and type X collagen in the cartilage of the collagen-induced arthritis (CIA) group was significantly increased and was decreased after Fufang Shatai Heji (STHJ) administration. The mRNA expression of aggrecan in the cartilage of the CIA group was significantly decreased and was increased after STHJ administration. All data are represented as mean ± standard deviation (SD). #, P<0.05, ##, P<0.01 *vs.* the control group; *, P<0.05, **, P<0.01 *vs.* the CIA group.

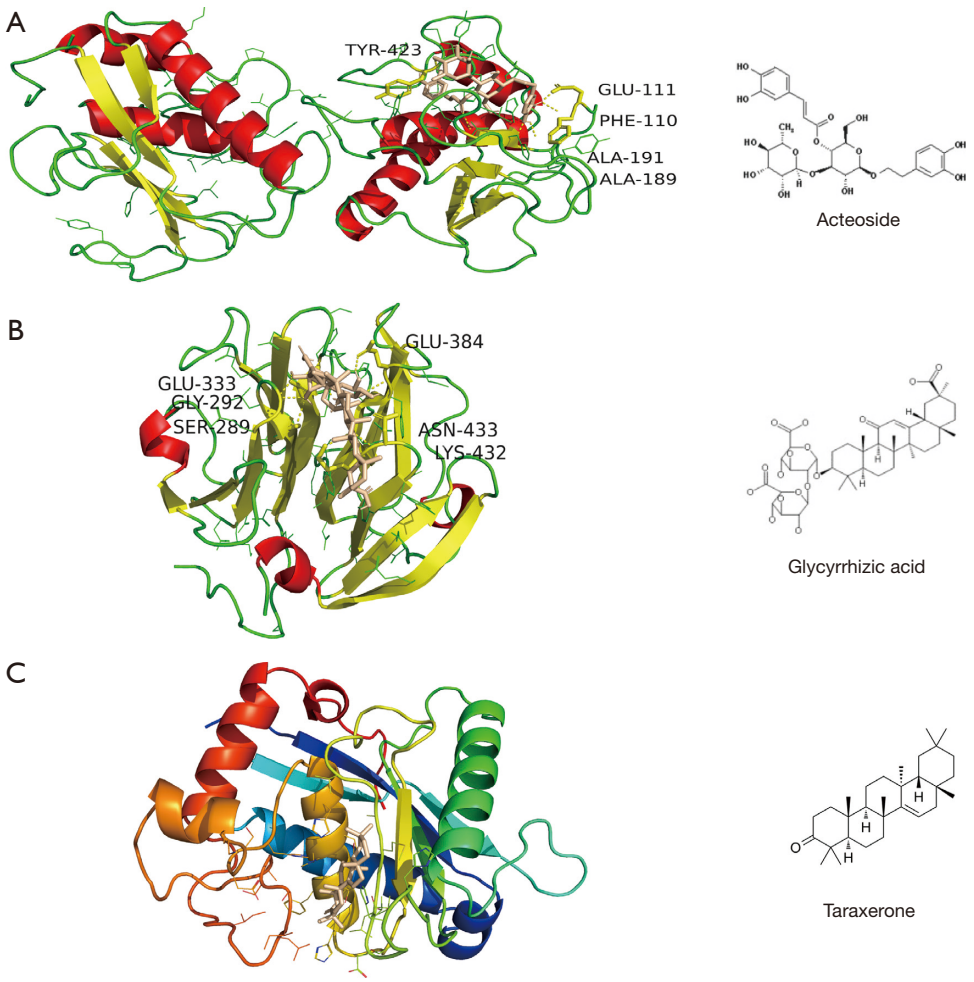

Figure 9 Three best docking results. (A) Acteoside; (B) glycyrrhizic; (C) taraxerone. The structure of the compound is represented by bars, the different branches of the protein are represented by different colors, and the hydrogen bonds are represented by yellow dashed lines. The hydrogen bond is formed in (A and B), and the position of the hydrogen bonds and amino acid residues are marked.

Table 2 Molecular docking

Chinese medicine	Chemical component	Potential targets		
		MMP-9	MMP-13	ADAMTS-5
Rehmannia glutinosa	Acteoside	Tyr423, Glu111, Phe110, Ala191 and Ala189	No	No
Glycyrrhiza Uralensis	Glycyrrhizic acid	No	Glu384, Asn433, Lys432, Glu333, Gly292 and Ser289	No
Radix Adenophorae	Taraxerone	No	No	No

this procedure, chondrocytes keep differentiating into hypertrophic chondrocytes. The hypertrophic chondrocytes are surrounded by a calcified cell matrix, coupled with the invasion of blood vessel, which contribute to the endochondral ossification (23). The unique marker of hypertrophic chondrocytes is type X collagen. Its synthesis is usually limited to the nutrient area of the skeletal growth plate, and mainly occurs in the late stage of cartilage ossification (24). Xu *et al.* found that the protein level of type X collagen increased in OA cartilage and was significantly associated with cartilage degeneration (25). In addition, MMP-13 can also promote the expression of type X collagen in cartilage ossification (26). Proteoglycan is the main ingredient of cartilage, and its loss is considered to be the key to cartilage damage. Aggrecan is the main proteoglycan and provides weight and elasticity to the cartilage (27). Degradation of aggrecan is an outstanding feature in RA. Degradation of aggrecan is usually mediated by cleavage of ADAMTS and MMPs at different sites (28). In this study, STHJ may inhibit cartilage destruction by regulating the expression of aggrecan and collagen X in cartilage.

MMPs are a kind of protease super families that are widely presented in numerous connective tissues and act a pivotal part in the degradation of ECM. The increased expression of MMPs is significantly associated with the progression of arthritis. Among various MMPs, MMP-9 and MMP-13 are the most important for the degradation of collagen. Collagen can be specifically degraded by MMP-9 which is a gelatinase. MMP-9 is extremely destructive and highly expressed in arthritis, and its decrease can improve the progression of arthritis (29,30). MMP-9 can also induce capillary to invade hypertrophic cartilage which lead to intraosseous ossification. Vu *et al.* found that MMP-9-deficient mice showed significant damage to endochondral ossification and a decrease in hypertrophic chondrocyte apoptosis during development (31). The expression of MMP-13 in osteoblasts and chondrocytes in the early stages of embryos is considered to be participated in endochondral ossification and bone remodeling. Transgenic mice overexpressing MMP-13 produced voluntary articular cartilage destruction, while MMP-13 knockout mice showed obvious defects in cartilage ossification (32). Therefore, MMPs are important factors in the maintenance of joint tissue homeostasis in RA. Doss *et al.* found that Ferulic acid, an important component of *Triticum aestivum*, *can* significantly reduce RANKL-induced osteoclast differentiation and down-regulated MMP-9 (33). Chen *et al.* found that Rosmarinic acid, an important component of Prunella vulgaris, can down-regulate the expression of MMP-13, etc. in chondrocytes induced by IL-1β (34). In this study, the mRNA expression of MMP-9 in cartilage of CIA mice increased, and obviously decreased after STHJ administration. Immunohistochemical analysis of knee and ankle joints showed that the expression of MMP-9 and MMP-13 increased in chondrocytes of CIA mice, and obviously decreased after STHJ administration. Therefore, STHJ may inhibit the degradation of collagen by regulating the expression of MMP-9 and MMP-13.

ADAMTS is a kind of aggrecanase that acts a pivotal part in the catabolism of ECM (35). ADAMTS is a large family of members, some of which are closely related to articular cartilage, such as ADAMTS-4, ADAMTS-5. ADAMTS-4 is considered to be the first enzyme to recognize aggrecan and may also take part in cartilage degradation (36). Liacini *et al.* found that tripterygium wilfordii Hook F (TWHF) inhibited the expression of ADAMTS-4 in chondrocytes thereby protecting cartilage (37). ADAMTS-5 is known as be the most important enzyme for the degradation of aggrecan (38). Glasson *et al.* found that the acceleration of the degradation of aggrecan of cartilage was not appeared in the ADAMTS-5 knockout mice under IL-1 stimulation as well as the absence of ADAMTS-5 prevented cartilage damage in the arthritis model (39). Hu *et al.* found that Rosmarinic acid, an important component of *Prunella vulgaris*, can inhibit the degradation of ECM in

osteoarthritis and inhibit the expression of ADAMTS-5 (40). In this study, the mRNA expression of ADAMTS-4 and ADAMTS-5 in the cartilage of CIA mice was increased, and obviously decreased after STHJ administration. Immunohistochemistry assay of knee and ankle joints also revealed that the expression of ADAMTS-5 increased in chondrocytes of CIA mice, but obviously decreased after STHJ administration. Thus, STHJ may inhibit the degradation of aggrecan by downregulating the expression of ADAMTS-4, ADAMTS-5, especially ADAMTS-5, thereby protecting against cartilage destruction.

Molecular docking is an effective tool in structural molecular biology and computer-aided drug research. The purpose of drug-protein docking is to predict the main binding modes of drugs and proteins with known three-dimensional structures. In addition, clarifying the relationship between the drug and the target is critical to the successful rational drug discovery. In the molecular docking study, it was found that a variety of active ingredients in STHJ have good binding activity with MMP-9 and MMP-13. Therefore, we believe that STHJ may achieve the anti-arthritis effect by effectively binding to MMPs and inhibiting their expression.

Conclusions

Overall, this study first proved that STHJ has an obvious protective effect on cartilage destruction in cartilage tissues in CIA mice. It mainly inhibited the degradation of ECM by suppressing the expression of MMP-9, 13 and ADAMTS-4, 5 in cartilage of CIA mice. In addition, molecular docking assay have shown that a variety of active ingredients in STHJ bind with potential target of RA which is worthy of detailed study in the further. Therefore, our study supposed that STHJ may be an effective drug in treating RA through cartilage protection.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://apm.amegroups.com/article/view/10.21037/apm-21-1765/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The Animal Ethics Committee of Shanghai Ninth People's Hospital approved all animal experimental protocol (No: SH9H-2021-A580-1), and the experiment complies with the institution's guidelines on the care and use of animals.

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